Monitoring *Phytophthora infestans* in Taiwan for Population Shifts that May Affect Late Blight Management Strategies

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ABSTRACT

Late blight caused by *Phytophthora infestans* is one of the most destructive diseases on tomato in Taiwan, especially in the eastern Taiwan. A total of 307 isolates of the pathogen collected by AVRDC- The World Vegetable Center during 2003 to 2005 were aggressive to both tomato and potato. All isolates were A1 mating type and all, except one isolate collected in 2003, were resistant to Metalaxyl. They were classified into ten physiological races by using six tomato differentials. All the isolates were analyzed by molecular genotype characterization. Most isolates (303/307) had dilocus allozyme genotypes 100/111 and 100/100, except 3 isolates were 100/122 and 100/100, for the loci coding the glucose-6-phosphate isozyme (*Gpi*) and peptidase (*Pep*), respectively. Mitochondrial haplotype analyses of polymorphic regions of the mitochondrial genome (mtDNA) were IIb. These characters, together with RG57 fingerprinting, indicated that these isolates belonged to the US-11 clonal lineage, containing 25% variant. There was only one isolates analyzed as 86/100 and 100/100 allozyme genotype for *Gpi* and *Pep*, Ib mtDNA haplotype, and belong to US-1 clonal lineage with RG57 fingerprinting. The results indicated that no new genotype of *P. infestans* immigrated into Taiwan during 2003-2005. Under the travel funding supported from Research and Scientific Exchanges Division, International Cooperation and Development, USDA, The principal leader Dr. T. C. Wang in AVRDC and the project collaborator, Dr. K. L. Deahl in the USDA-ARS Vegetable Lab hold meetings in Taiwan and USA for exchanging new scientific information and technology, setting up the laboratory procedures in AVRDC, and planning further collaboration. Dr. Deahl and his assistant Frances Perez also provided the kind assistance to hold a training workshop for molecular characterization of *P. infestans* isolates for AVRDC's and Taiwan researchers in 2004. Increasing percentage of the US-11 variant indicated that genomic diversity of the pathogen are changing quickly although sexual recombination was not yet detected. Therefore, it is important to monitor the population change of the *P. infestans* and develop the integrated management strategy for the disease control continuously.

Key words: *Phytophthora infestans*, Physiological race, Mating type, Metalaxyl, Genotype characterization

INTRODUCTION

Late blight, caused by *Phytophthora infestans* (Mont.) de Bary, is a highly destructive disease affecting both tomatoes and potatoes. The pathogen is most active during cool, moist weather conditions. The disease has increased in severity in many parts of the world in recent years. This has been associated with the migration of new and more aggressive populations of the pathogens (Spielman *et al.*, 1991). Since 1998 severe late blight epidemics have occurred in Taiwan both on tomato and potato crops concomitant with the appearance of a new genotype US-11, of the pathogen (Ann and Chang, 2000). It is highly plausible that the new genotype was introduced on imported table potatoes during 1997-98 growing season (AVRDC, 1999; Ann and Chang, 2000; Deahl *et al.*, 2002). By 2000 the new genotype had almost completely displaced the old US-1 genotype, which all Taiwan isolates collected during 1991-97 (Deahl *et al.*, 2002;). The new US-11 genotype appears to be more aggressive on tomato and potato. Moreover it carries resistance to metalaxyl, a fungicide on which Taiwan tomato farmers had relied for several years to manage the disease (AVRDC, 1999; Ann and Chang, 2000). Fortunately, US-11 is of the A1 mating type as was the old US-1 genotype. And the A2 mating type of *P. infestans* has not yet migrated into Taiwan. The introduction of the A2 mating would increase genetic recombination through sexual reproduction locally, and has been a concern for local quarantine.

AVRDC has been breeding for tomato late blight resistance since 1994 utilizing resistant sources from *Lycopersicon pimpinellifolium*, accession L3708, and *L. hirsutum*, accession LA1033, which were selected for resistance against the old genotype US-1 (AVRDC, 2000; Chungwongse *et al.*, 2002). Thus far, these resistance sources also hold up against the new genotype US-11 of *P. infestans* until 2004. However, the resistance of AVRDC's resistant lines was broke-down due to the occurrence of new races (Race T1, 3, & T1, 2, 3, 4) during 2003 (AVRDC, 2004). Therefore, there is a need to constantly monitor the population for possible emergence of new physiological races and for the introduction of additional genotypes, in particular those that may be of the A2 mating type. Knowledge of this kind is essential to develop appropriate breeding approaches and the overall disease management strategies.

This project was designed to assess occurrence of and severity of late blight island-wide in tomato and potato crops through seasonal survey in Taiwan during 2003 to 2005, and to monitor for changes in the late blight pathogen, *P. infestans* for the management strategies of late blight control and for the development of quarantine regulations by the Bureau of Animal and Plant Health Inspection and Quarantine, Council of Agriculture. This project was also planned to invite Dr. Kenneth L. Deahl, Plant Pathologist in USDA-ARS Vegetable Lab. at Beltsville, and his assistant Ms. Frances Perez to visit Taiwan and AVRDC–The World Vegetable Center to present seminars for discussing and exchanging current information on local and international dissemination of new isolates of *P. infestans*

with Taiwan scientists and to be resource persons for the training workshop of "molecular genotype characterization on *P. infestans*" at AVRDC for transferring the advanced molecular bio-technology with the researchers in universities and research institutes.

MATERIALS AND METHODS

Island-Wide Surveys on Late Blight of Tomato and Potato Crops in Taiwan, and Pathogen Isolation

Island-wide surveys on late blight of tomato and potato crops were made in most counties in Taiwan to assess late blight occurrence, severity, and to collect infected host tissues. Pathogen isolates were isolated from diseased samples by cutting out host tissue pieces (3-4mm^2) from the margins of lesions, surface sterilizing, and transferring to petri plates containing rye A agar amended with antibiotics, including 100 ppm ampicillin, 100 ppm nystatin, and 50 ppm rifampicin (Caten and Jinks, 1968). Pure isolates were preserved on autoclaved rye grain with water at 16-20°C.

Physiological Race Identification

A total of 158 isolates were artificially inoculated with zoospore/sporangia suspension (5 x 10^4/ml) on six tomato differentials: TS19 (*Ph*$^+$); TS33 (*Ph*-1); W.Va.700 (*Ph*-1, 2); CLN2037B (*Ph*-1, 2, 3); L3708 (*Ph*-1, 2, 3, 4), and LA1033 (*Ph*-1, 2, 3, 4, 5) for the first 24 hr at 100% RH and 20±2°C without light, thereafter, maintained at 60-95% RH and 20±2°C with a 14-hr light (70μE.m^{-2}.5^{-1}) period per day. The pathogenicity of each isolates was evaluated 7 and 10 days after inoculation (AVRDC, 1999). Based on the mean disease severity rating of differentials, isolates could be grouped (Vartanian and Endo, 1985; AVRDC, 1999). The designations of these races are derived from the genes the races defeat.

Host Specialization

All isolates were tested for their aggressiveness to potato by inoculating "Kennebec" potato plants at 49 days after planting. Susceptible tomato variety FMTT-22 was arranged as the control. The inoculation method, incubation conditions, and disease severity rating were the same as above-mentioned.

Assessment of Metalaxyl Sensitivity *in Vitro*

Metalaxyl sensitivity of isolates was determined by measuring colony radial diameter twice at 90° angle for each isolate colony size after cultured for 14 days at 20°C on rye A agar medium amended with 100ppm of metalaxyl (Pappas, 1985). Two measurements were averaged as colony diameter. Three replications of each isolate were averaged for the analysis by Duncan's Multiple Range Test (P≤0.05) for the comparison. Sensitive isolates, Pi39, 43, 64 and 75 were arranged as the control.

Determination of Mating Type

Mating type of *P. infestans* isolates was determined by pairing each isolate with known A1 (isolate P991) and A2 (isolate P731) mating type standard cultures of *P. parasitica* on rye A agar; paired cultures were incubated at 20±1℃ in the dark for 10-14 days. Isolate that formed oospores/ oogonia when paired with known A2 mating type were designed A1 mating types; those forming the sexual reproductive structures with known A1 mating type were designed as A2 mating type (Hohl and Iselin, 1984).

Molecular Genotype Characterization of *P. infestans*

Molecular genotype characterization was followed by the global marker database (Forbes *et al.*, 1998). Genotypes at two polymorphic allozyme loci, *Gpi* and *Pep*, were determined using the protocols developed by Goodwin *et al.* (1995). Enzymatic activities were revealed following electrophoresis and staining with the appropriate agar overlays. The mtDNA haplotypes of isolates were determined by PCR-RFLP using a method modified from Griffith and Shaw's protocol (1998). P2 and P4 DNA were amplified by oligonucleotide primers, F2/R2 and F4/R4, and then restricted by *Msp*I and *Eco*RI, respectively. Digested DNA patterns were resolved by electrophoresis run at approximately 10V cm^{-1} for 1-1.5 hours and visualized using a UV transilluminator at 254 nm. DNA fingerprinting using the moderately repetitive probe RG57 was carried out as described by Goodwin *et al.* (1998). The genomic DNA of *P. infestans* was transferred to Hybond N+ Nylon membrane and then hybridized by the RG57 probe, labeled with fluorescein. All of the new isolates were also sent to USDA-ARS Vegetable Lab at Beltsville for molecular genotype characterization for the comparison with the isolates collected from other countries in the world.

Exchange of Scientific Information and Technology

Under the travel funding supported from Research and Scientific Exchanges Division, International Cooperation and Development, USDA, the project was designed to hold meetings in both Taiwan and USA for exchanging new scientific information and technology, setting up the laboratory procedures in AVRDC and planning further collaboration. AVRDC and Dr. Deahl also cooperated to hold a training workshop for molecular characterization of *P. infestans* for the research staffs at AVRDC and local research institutes.

RESULTS

Island-Wide Surveys on Late Blight of Tomato and Potato Crops in Taiwan and Pathogen Isolation

Late blight was continually occurred, devastated and caused severe economic losses in highland production areas and eastern Taiwan during spring season due to lower night temperature and higher relative humidity. A total of 307 isolates of *P. infestans* were isolated from diseased tomato and potato samples collected from 14 counties in Taiwan.

Identifications of Physiological Races, Host Specialization, Metalaxyl Sensitivity, and Mating Type

All the isolates were A1 mating type, resistant to metalaxyl (except one collected in 2003), and aggressive on both tomato and potato without host specificity. A total of 158 isolates collected in 2004 and 2005 were grouped into ten physiological races consisting of: Race T1; T1,2; T1,3; T1,2,3; T1,2,5; T1,3,5; T1,2,3,4; T1,2,3,5; T1,3,4,5; and T1,2,3,4,5 (Table 1). Race T1,2,3 seems the predominant one.

Molecular Genotype Characterization of *P. infestans*

Most isolates (303/307) had dilocus allozyme genotypes 100/111 and 100/100, except 3 isolates were 100/122 and 100/100,

for the loci coding the glucose-6-phosphate isozyme (*Gpi*) and peptidase (*Pep*), respectively. Mitochondrial haplotypes evaluated through PCR amplification and RFLP analysis of polymorphic regions of the mitochondrial genome (mtDNA) were IIb mtDNA haplotype. These characters, together with RG57 fingerprinting, indicated that these isolates belonged to the US-11 clonial lineage, containing 25% (77/306) variant. There was only one isolates analyzed as 86/100 and 100/100 allozyme genotype for *Gpi* and *Pep*, Ib mtDNA haplotype, and belong to US-1 clonal lineage with RG57 fingerprinting (Table 2).

Exchange of Scientific Information and Technology

The principal leader, Dr. Tien-cheng Wang and Assistant Mr. Zong-ming Sheu were invited by project collaborator, Dr. K. L. Deahl, to visit USDA-ARS Vegetable Lab at Beltsville, MD, USA during September 12th to 25th in 2003 to exchange information of population shift of *P. infestans* in the world and to learn the molecular genotype characterization technology; AVRDC's pathologist Dr. J. F. Wang met with Dr.

Table 1. Designation of putative physiological races of *Phytophthora infestans* isolates collected from tomato in Taiwan from 2004 to 2005.

Race	TS19 (*Ph*+)	TS33 (*Ph*-1)	W. Va. 700 (*Ph*-1,2)	CLN2037B (*Ph*-1,2,3)	L3708 (*Ph*-1,2,3,4)[1]	L1033 (*Ph*-1,2,3,4,5)[2]	No. of Isolates
T1	S	S	R	R	R	R	13
T1,2	S	S	S	R	R	R	20
T1,3	S	S	R	S	R	R	14
T1,2,3	S	S	S	S	R	R	66
T1,2,5	S	S	S	R	R	S	1
T1,3,5	S	S	R	S	R	S	2
T1,2,3,4	S	S	S	S	S	R	16
T1,2,3,5	S	S	S	S	R	S	17
T1,3,4,5	S	S	R	S	S	S	2
T1,2,3,4,5	S	S	S	S	S	S	7

[1,2]Gene(s) conditioning late blight resistance in this accession are not yet characterized.

Table 2. The genotype characteristics of *Phytophthora infestans* isolates collected in Taiwan during 2003 to 2005.

No. of isolates collected	RG57[1]			Gpi/Pep[3]			mtDNA haplotype[4]	
	US-1	US-11	US-11v[2]	86/100 92/100	100/111 100/100	100/122 100/100	Ib	IIb
307	1	229	77	1	303	3	1	306

[1]DNA fingerprint analysis using RG57 probe.
[2]Variant that misses one band (band 5 or 10) and/ or appears one band (band 12 or 18) on fingerprinting pattern.
[3]Allozyme genotype analysis.
[4]Mitochondrial haplotyping through PCR and RFLP analysis.

Deahl in the American Phytopathology Society 2004 Annual Meeting hold at Anaheim, California during August 31st to September 1st to exchange the recent research results and discuss the international dissemination information of *P. infestans*. Furthermore, they also confirmed the detail of the train workshop that would be hold at AVRDC in October 2004.

Project collaborator Dr. Deahl, Assistant Ms. Frances Perez, and former AVRDC plant pathologist Dr. Lowell L. Black were invited to visit AVRDC and Taiwan by Dr. Thomas A. Lumpkin, Director General of AVRDC during October 21st to 30th in 2004. They visited Nantou County on October 24 and observed destructive late blight in many tomato fields. Dr. Deahl presented the seminar entitled "Population changes in *Phytophthora infestans* in the USA and Canada associated with the appearance of resistance to metalaxyl and the A2 mating type" at AVRDC on October 25. Dr. Deahl was also invited as a resource person of the training workshop "Molecular genotype Characterization" hold by AVRDC during October 25th to 27th. Dr. Deahl and Ms. Frances advised AVRDC on setting up laboratory procedures for molecular genotype characterization of the isolates during the workshop. A total of 14 participants from local institutes, universities and AVRDC attended the training workshop. Dr. Deahl and Dr. Black visited AVRDC's scientists to understand their on-going research activities and also to discuss the possibility of future collaboration.

Drs. Deahl, Black and Ms. Frances accompanied with Dr. T.C. Wang visited Department of Plant Pathology, National ChungHsin University, and Department of Microbiology and Plant Pathology, National Taiwan University to present the seminars on October 28th and October 29th, respectively. They reviewed with Taiwan professors, scientists and graduate students on methodologies for characterizing *P. infestans* isolates and exchanged the information of advanced biotechnology. Their visit improved the communication of new message and the exchange of advanced biotechniques. They also visited Bureau of Animal and Plant Health Inspection Quarantine (BAPHIQ) to meet with senior officers Drs. Huang, Tze-Chung and Kuo, Ker-cheng for exchanging the experience on recent international dissemination of *P. infestans* and quarantine regulations, which could be valuable for the development of quarantine strategies.

Mr. C. H. Chen was invited and visited to USDA-ARS Vegetable Lab during November 15th to 25th in 2005, to update techniques on molecular characterization, especially the technique of rapid detection A2 mating type of *P. infestans*, which is very helpful for the quarantine.

Dr. T. C. Wang and Assistant Z. M. Sheu and C. H. Chen attended the American Phytopathology Society (APS) 2005 Annual Meeting in Austin, USA, during July 30th to August 3rd, presented a paper related to "The variation of race composition of *Phytophthora infestans* in Taiwan during 1991 to 2004", and exchanged information with scientists in APS. Then they visited Dr. K. L. Deahl and his post-doctor Dr. Stefano Costanzo at USDA-ARS Vegetable Lab. in Beltsville during August 4th to 8th to update molecular genotype characterization techniques, discuss on the project results, and plan for future collaboration.

CONCLUSIONS

Late blight was epidemic and caused severe yield loss in the eastern Taiwan as well as in highland areas where were lower temperature and higher relative humidity in the growth season of tomato and potato during 2003-2005. All isolates collected in this study were very aggressive and resistant to metalaxyl. That maybe why the farmers were difficult to get effective disease control by fungicides application. The development of an integrated disease management strategy will be very important and urgent.

Ten physiological races of *P. infestans* were identification from 130 isolates. Race T1,2,3 seems the predominant race during 2004 to 2005. It is critical, therefore, to continue monitor the occurrence of new races of *P. infestans* in the future.

The results of the mating type identification also indicated that the A2 mating type of *P. infestans* has not yet found into Taiwan. If the A2 mating type was migrated into Taiwan, it will become a complex problem due to its sexual reproduction. Mating type is very important to the quarantine regulations of BAPHIQ, the rapid detection of mating type will be useful to improve the quarantine efficiency.

The results of the molecular genotype characterization showed that new genotype US-11 has almost completely displaced the old population US-1. No new genotypes were identified during 2003 to 2005. However, increasing percentage of the US-11 variant indicated that genomic diversity of the pathogen is changing quickly although sexual recombination was not yet detected. Therefore, it is necessary to monitor the population shifts of the *P. infestans* and develop the integrated management strategy for the disease control continuously.

This successful collaboration activity not only documented the population shifts of *Phytophthora infestans* in Taiwan for the important references of BAPHIQ to develop the quarantine strategy but also improved the communication of new message and the exchange of advanced biotechniques between AVRDC and USDA-ARS Vegetable Lab. This will be a good example for future collaboration.

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